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Endogenous locomotor activity rhythm of two sympatric species of Talitrids (Crustacea, Amphipoda) from a sandy beach of Tuscany, Italy

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Abstract. The biological clock of living organisms, which is an evolutionary effect of their temporal adaptation to the environment, is known to be entrained by natural periodic variables; the behavioural and physiological response to these variables can be traced by laboratory recordings under constant conditions. Adult individuals of two sympatric talitrid species (*Talitrus saltator* and *Orchestia gammarellus*), inhabiting different niches of the same coastal ecosystem in the Maremma Regional Natural Park (Grosseto, Italy), were studied for their locomotor activity rhythm. Recordings were carried out by an automatic electronic apparatus and lasted for 21 days in constant darkness and controlled temperature and humidity. At the end of the recording sessions, the cephalic length and antennal tagmas of the tested specimens were measured and sex determined. Statistical analysis showed a relevant difference in rhythm expression between the two species, which was related to their different physiology and ecology and highlighted a differentiated adaptation to terrestrial life of the two species. *O. gammarellus* showed a longer mean period and *T. saltator* a higher mean definition, confirming a finer use of the biological clock in the latter species. This study leads to the general conclusion of a higher dependence of *T. saltator* from the circadian cycle as compared to *O. gammarellus*. This behaviour can be directly linked with the colonisation of the aphytoic sandy beach by *T. saltator*, while *O. gammarellus* in this area occupies the vegetation of the dune slack, accordingly showing a more variable rhythm, less dependant on daily cycle.

Key words: Sandy beach, biological rhythm, sympatric species, *Talitrus saltator*, *Orchestia gammarellus*

Résumé. ***Rythme d'activité locomotrice endogène de deux espèces sympatriques de Talitridés (Crustacea, Amphipoda) d'une plage sableuse de la Toscane, Italie.*** Les horloges biologiques des organismes vivants sont le résultat de l'évolution et de l'adaptation temporale à l'environnement. Une des caractéristiques les plus importantes des horloges biologiques est celle de permettre la synchronisation des organismes aux cycles des variables environnementales. La réponse, soit physiologique soit comportementale, ainsi entraînée, est observée en conditions constantes de laboratoire. Les rythmes d'activité locomotrice ont été étudiés chez des individus appartenant à deux espèces sympatriques (*Talitrus saltator* et *Orchestia gammarellus*), colonisant différentes niches du même écosystème côtier à l'intérieur du Parc Régionale de la Maremma (Grosseto, Italie). Un système automatique d'enregistrement pendant 21 jours a été utilisé en conditions d'obscurité continue à des température et humidité contrôlées. A la fin des sessions expérimentales de chaque individu testé, la longueur du cephalon a été mesurée, le nombre de segmentes des antennes a été compté et le sexe identifié. L'analyse statistique a montré une différence significative dans l'expression des rythmes entre les deux espèces, qui a été mise en relation avec la différence de leurs physiologie et d'écologie et met en évidence une adaptation différenciée à la vie semi-terrestre de ces deux espèces. La période moyenne d'activité en libre cours de *O. gammarellus* est plus longue que celle de *T. saltator*, qui a montré une meilleure définition moyenne de la période. Ces résultats confirment une utilisation plus fine de l'horloge biologique par la seconde espèce, qui en conclusion a une dépendance plus stricte de rythmes circadiens. Cette différence de comportement peut être mise directement en relation avec la colonisation par *T. saltator* de la zone aphytique de la plage. En revanche, *O. gammarellus* colonise la végétation de la dépression de l'arrière dune, et en conséquence montre un rythme plus variable et moins lié au cycle journalier.

Mots clés : Plage sableuse, rythme biologique, espèces sympatriques, *Talitrus saltator*, *Orchestia gammarellus*

INTRODUCTION

Talitrid amphipods are semi-terrestrial crustaceans with a cryptozoic or burrowing lifestyle. They have a breathing system composed by gills and a permeable cuticle without protective waxes against water loss (Williamson 1951, Williams 1995). They colonise habitats with the common feature of providing a humid microclimate that is of fundamental importance for their survival by preventing desiccation (Hurley 1968, Spicer *et al.* 1987). Talitrid species inhabiting coastal zones are well known in literature for their nightly locomotor activity rhythm (Bregazzi & Naylor, 1972; Williams, 1980, 1983), a behavioural adaptation to the light-dark cycle that allows them to avoid dehydration during daytime. For the species *Talitrus saltator*, variability in the expression of the locomotor activity rhythm is documented depending on geographical distribution (Williams 1980, Nasri-Ammar & Morgan 2006, Rossano *et al.* 2008, 2009) and seasonality (Nardi *et al.* 2003), thus highlighting a certain degree of variation within species. Within the family Talitridae a higher degree of variation between species was observed reflecting adaptation of species to various environments (Williams 1983, Rossano *et al*. 2009). Anyway few comparisons among the rhythms of sympatric talitrid species that could result in relevant behavioural diversities linked to their ecological habits were carried out till now (Williams 1983, Fallaci *et al*. 1999). The aim of this study was to analyse the locomotor activity rhythms of two sympatric species living on an Italian sandy beach within a

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protected area (the Maremma Regional Natural Park) to test the hypothesis that two species of the same family, sympatric in the same restricted area, exploit the most suitable microhabitat and colonise separate niches by exhibiting different behaviours.

MATERIAL AND METHODS

The amphipods were collected in the Maremma Regional Natural Park (Grosseto, Italy), in a beach with restricted access to the south of Collelungo beach (42° 37' 48" N, 11° 04' 52" E). The beach was about 60m width from waterline to the first pioneer vegetation, backed by a small dune system and a dune slack, where brackish water remained during winter and spring.

We collected *Talitrus saltator* specimens mainly on the beach between the drift line and the dunes, and *Orchestia gammarellus* further from the waterline in the dune slack mainly under *Juncus acutus* bushes and under stranded woods. The two sites of collection were in some places overlapping and some specimens of *T. saltator* were observed on the dune. Live samples of adult individuals (size>8 mm, Williams 1978) were transferred to the Department of Evolutionary Biology at the University of Florence in isolated containers, and within a few hours from collection each single animal was placed in an annular recording chamber made of opaque PVC (diameter of 10.5 cm and height of 13 cm) provided with an infrared ray recording system (Scapini *et al.* 2005). Each recording chamber was connected to a logger that downloaded data every 20 minutes. The recording apparatus and software had been provided by the workshop of the School of Biosciences of the University of Birmingham (UK). Specimens were housed singly in the recording chambers with humid freshly collected substratum (sand for *T. saltator* and sand mixed with soil for *O. gammarellus*). "Tetramin" dry fish food for tropical aquaria was provided on a small piece of filter paper. Each recording session lasted 21 days in continuous darkness and at a constant temperature of 18±1°C, with the aim of producing free-running conditions and assessing the endogenous component of the locomotor activity rhythm (Aschoff 1960). Three recording sessions were performed in June, July and August, and the two species were tested at the same time during each session. At the end of each recording session animals were preserved in separate tubes with alcohol 75% and sex, cephalic length and the number of tagmas of the second antennae (as proxy of age, Williams 1987) were estimated by microscope observations. Presence of penes for males and oostegites for females confirmed adult life stage in all individuals for *T. saltator* (following Williams 1978) and this criterion was considered extendible to *O. gammarellus* also.

Actograms representing the individual locomotor activity pattern were obtained by the Chart software package version 35 (D.D.Green, University of Birmingham, UK). Subsequent periodogram analysis was performed using the program based on the method of Dorscheidt and Beck (Harris & Morgan, 1983).

The percentage of survival, number of active animals and number of animals showing periodicity were estimated for each sample; the mean circadian period (τ) and the signal-to-noise ratio (SNR, as a measure of definition) were calculated for each individual. The definition of the period (SNR) was calculated as ratio between the correlation ratio of the periodogram and the 95% probability line in the periodogram analysis, and was used as a measure of the definition of the rhythm. All the periods analysed in these experiments were significant at $p < 0.05$ level.

RESULTS

A good rate of survival was recorded for both species (Table I) indicating that animals were housed under suitable conditions. Not all the tested animals were used in the analysis, since some had died during the recording sessions and some recording chambers had failed in recording the locomotor activity. On those animals that were considered in the analysis a high percentage (94% for *T. saltator* and 90% for *O. gammarellus*) was active for more than 15 days over the 21 of recording (Table I). Over these active animals a significantly lower number of individuals of *O. gammarellus* exhibited a circadian period compared to *T. saltator* (Pearson's chi-square test with Yates' continuity correction: 5.3378, df=1, p=0.0209).

T. saltator had a shorter (closer to the 24 h) and less variable mean period than *O. gammarellus* (Table I), however this difference was not significant (Wilcoxon rank-sum test with correction, Z=1.607, p=0.108), while the definition of the period of *T. saltator* was significantly higher (Wilcoxon rank-sum test with correction Z = 2.8745, p=0.004).

In Figure 1a,b the period definition (SNR) was plotted against the length of the period for the two species. For *T. saltator* a higher concentration around the mean and higher definition are evident (Fig. 1a), while for *O. gammarellus* the cloud of points is more dispersed along the y-axis and shows a more flattened pattern, indicating a general lower definition of the period.

Regression analysis was performed between the period (τ) or its definition (SNR) and the cephalic length or number of antennal tagmas; however no result was significant (F-statistic, always p>0.05). Comparisons were performed for period and definition between males and females within the same species and between species, however neither this comparison was significant (Wilcoxon rank-sum test, always p>0.05). Anyway in *T. saltator* a trend was visible for period definition, with females showing a SNR slightly higher than males, whereas in *O. gammarellus* an opposite trend was observed (Table II).

DISCUSSION

A previous published research was performed in the same area by the authors, on three species of talitrids colonising the coast and the inland (Rossano *et al.* 2008). By focussing the attention on the two species of *Talitrus saltator* and *Orchestia gammarellus* of this previous study

(Table III), it is possible to make some comparisons and formulate some hypothesis. These previous experiments were conducted with the same protocol, comparable samples and during the same season (summer) of the present study. The survival of the animals in the recording apparatus for the present study and for the previous one was always near to the 100%, proving the suitable conditions in which the experiments were conducted.

Table I: Comparison of the two species

Species	N_1	Survival	N_2	Active	Periodic [a]	N	Mean τ	Mean SNR [b]
T. saltator	61	97%	48	94%	96%	43	24h19’ ± 6’	0.503 ± 0.062
O. gammarellus	56	91%	52	90%	77%	36	24h41’ ± 12’	0.286 ± 0.046

N_1: recorded animals; N_2: analysed animals; active: percentage of active individuals calculated on N_2; periodic: percentage of periodic individuals calculated on active animals; N: number of periodic individuals; mean rhythm period (τ), mean definition (SNR), and relative standard error

[a] Pearson's chi-square test with Yates' continuity correction: 5.3378, df = 1, $p = 0.0209$

[b] Wilcoxon rank-sum test with correction, Z = -2.8745, $p = 0.004$

Table II: Comparison of mean period and definition for males and females within the two species

Species	Sex	N	Mean τ	Mean SNR
T. saltator	m	19	24h 20' ± 5'	0.482 ± 0.104
	f	24	24h 18' ± 9'	0.520 ± 0.077
O. gammarellus	m	16	24h 46' ± 19'	0.293 ± 0.051
	f	19	24h 39' ± 15'	0.282 ± 0.077

Sex: males (m) and females (f); N: number of individuals; mean period (τ), mean definition (SNR), and relative standard error. Wilcoxon rank-sum test, always $p > 0.05$

Table III: Comparison of two species, four populations, from the Maremma Regional Park (cited from Rossano *et al*., 2008)

Species	N_1	Survival	N_2	Active	Periodic	N	Mean τ	Mean SNR
T. saltator beach	69	97%	40	95%	74%	28	24h13' ± 04'	0.456 ± 0.059
T. saltator canal	78	95%	61	70%	93%	40	24h12' ± 05'	0.484 ± 0.059
O. gammarellus river	62	85%	49	53%	38%	10	24h04' ± 19'	0.186 ± 0.041
O. gammarellus cave	78	94%	78	83%	46%	30	24h31' ± 18'	0.151 ± 0.024

N_1: recorded animals; N_2: analysed animals; active: percentage calculated on N_2; periodic: percentage calculated on active animals; N: number of periodic individuals; mean rhythm period (τ), mean definition (SNR), and relative standard error.

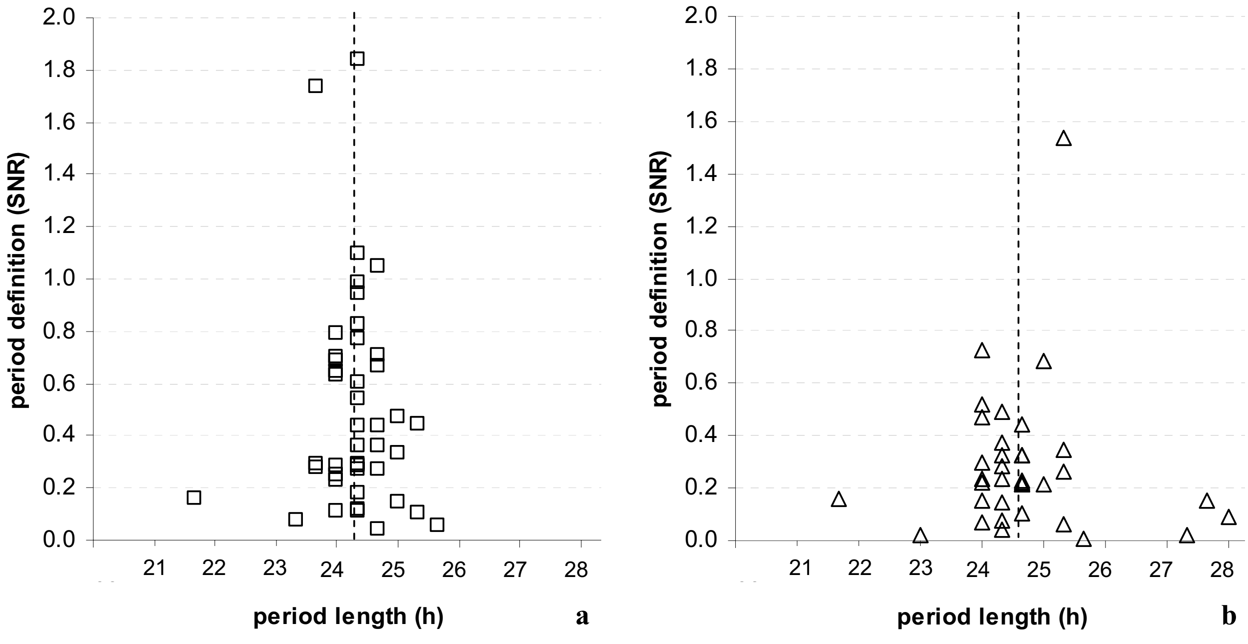

Figure 1: Scatter plots of the period definition: a) *Talitrus saltator*; squares, individual means; b) *Orchestia gammarellus*; triangles, individual means. The mean period length is represented as a dotted line.

T. saltator samples were collected on the same beach (Collelungo) about 1 km to the north (5 km from the Ombrone river mouth) of the locality where this study was conducted, an area without dune slack and with higher touristic pressure, being near to the beach access of one of the touristic paths of the Park. The percentage of active individuals of *T. saltator* from C6 is comparable with data already presented for C5. Animals from C5 resulted less periodic (74%) than those from C6 (96%) and this can be due to a different level of disturbance in the area. In the 2008 study a second sample of *T. saltator* was collected on a temporary pool at the end of a canal at the Ombrone river mouth and those animals exhibited a clearer periodicity than animals from C5. This second sample showed a percentage of periodic animals and a period definition more similar to C6 than to C5. Probably the difference observed may be attributed to the different tourist frequentation of the study areas, as the expression of rhythmic behaviour may be disturbed by trampling.

The most interesting result from this comparison is that *O. gammarellus* from the dune slack (Tables I & III) was very periodic (77%) as compared to the two samples from the previous study (Tables I and III, 38% for the river bank and 46% for the cave entrance), the activity period was longer and the period definition was higher, almost double of the definition of the cave entrance sample (Tables I & III). It stays that *T. saltator* exhibits a clearer and generally shorter (closer to 24 h) period than *O. gammarellus* both in the present study as in 2008 results (with a higher percentage of periodic animals and a higher definition in *T. saltator*). We may hypothesise that the cryptozoic species *O. gammarellus* analysed in the present study colonises a relatively drier environment, where the only shelters are *Juncus acutus* bushes and sparse woods; under those conditions the need of expressing a circadian rhythmicity increases. On the other hand, both the populations tested in 2008 came from humid habitats, the banks of a river and wet mud at a cave entrance.

The data relative to rhythm expression hived off for sex, even if only showing a trend, seem to be of great interest. The trend for *T. saltator* females is to have a more defined rhythm, which may be explained by the ecology of the species that doesn't have a sheltered area were to feed or mate, but is obliged to leave the refuges within the sand to carry out these activities. Therefore females that frequently carry eggs or embrios in summer may be more sensitive to dehydration and need to express more precise circadian rhythms. The opposite happens for *O. gammarellus* that lives sheltered in *Juncus*. Also in this species during reproductive periods, females are more sensitive to dehydration than males, as they need to osmoregulate to preserve eggs from desiccation (Morritt & Spicer 1996), It is possible to hypothesise that females do not frequently leave the shelter of the bushes, where conditions are more suitable and where they can feed on rotten vegetation. On the contrary, males would need to move from a sheltered position to another to mate or to disperse, and therefore would need a more precise activity cycle to avoid finding themselves in unsuitable dry conditions during the day. In talitrids behavioural adaptations are more important than physiological ones (Morritt 1998).

On the whole, this study confirms the importance of different adaptations depending not only on species but also on populations, whose response to the environment, within the range of capabilities of each species, are strictly linked to the limits the environment itself poses to the survival of each individual.

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